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Effect of melatonin treatment on fertility, fecundity, litter size and sex ratio in ewes

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Abstract. *The current study was designed to investigate the effects of administering melatonin alone on reproductive performance and inducing estrus in ewes during anestrous season. In the experiment a significant effect of breed and time of melatonin implants treatment was recorded for fertility, litter size (number of lambs born per ewe) and fecundity in the three breeds under study. Melatonin treatment increased the fertility rate significantly in the two spring treatment periods: March - ewes Group I and IV), and April – ewes Group II in the Pleven Blackhead x Awassi and Ile de France flocks. Regarding litter size, the melatonin-treated ewes recorded a significantly higher number of lambs born after treatment in March in the meat breed. The lower result (115% fecundity) was obtained in experimental Group III (Awassi). The melatonin implants improved the fertility in the ewes when inserted in spring (II-94,5%; III – 93,26%; IV – 91,42%). The highest increment in the number of lambs born per ewes treated in these three breeds was obtained in April - Farm 2. The litter size frequency for the three breeds under study were: (0) 5%, (1) 57,964%, (2)36%, (3) 1,036%. Of particular interest was the frequency of litter size and sex ratios of newborn lambs.*

Keywords: ewes, melatonin, lamb sex ratio, ewes litter size

Introduction

Sheep and goats are important in development because of their ability to convert forages and crops and household residues into meat, skins and milk. The economic importance of each of the products varies between regions, especially in the agricultural countries (FAO, 1981).

Species that use photoperiod to synchronize breeding activity are commonly classified in two different categories; long-day breeders or short-day breeders. Species in the first group, which include the ferret, the hedgehog and the horse, enter their breeding season after the winter solstice when the day length increases. Species in the second group, such as deer, goats and sheep, become sexually active in response to decreasing day length in the late summer to early autumn. Of the many environmental variables available, photoperiod is the most commonly used synchronizing agent (Karsch et al., 1984) because unlike other climatic variables such as temperature and rainfall, the seasonal cycle of day length is constant between years. Reproductive seasonality in ewes is characterized by changes in behavioral, endocrine, and ovulatory patterns (Epstein, 1985; Rosa and Bryant, 2003). Seasonal reproduction in sheep is mainly regulated by photoperiod through melatonin secretion along with other environmental factors such as temperature, nutrition, and social relationships (Arendt, 1998).

Melatonin is the main hormone secreted by the pineal gland and is by far the most extensively studied pineal compound. In several species, melatonin can also be synthesized in other organs such as the retina, intestine and salivary glands (Vivien-Roels and Pévet, 1983) but in most mammals the pineal gland accounts for almost all the melatonin in circulation (Yellon et al., 1992). Melatonin is the pineal gland hormone produced during dark hours and appears to be responsible for the regulation of the effects of daily photo-period on reproduction, triggering the change from seasonal anestrus to the ovulatory period. Daily administration of exogenous melatonin to anestrus ewes advances the breeding season. Prolonged administration of exogenous melatonin during the anestrous season leads to an early onset of the breeding season (Misztal et al., 2002). Melatonin implants have the ability to advance and condense the breeding season in ewes from a wide range of breeds with different patterns of seasonality (Abecia et al., 2005). The treatment must begin within the effective period, which depends on the normal date of seasonal onset of estrus for the breed. Haresign et al., (1990) suggested that the optimum implantation of melatonin is around 60 days before the normal onset of estrus for a given breed. The response of ewes to melatonin treatment depends on breed (Abecia et al., 2006), season and location. The objective of this experiment was to determine fertility, litter size and fecundity in the three sheep breeds after treatment with melatonin implants.

Material and methods

The experiment was conducted using adult, cycling, non-pregnant fat-tailed Awassi, crosses F_2 with Bulgarian local sheep breed (Pleven Blackhead x Awassi) and Ile de France breed. The interval between the previous parturition and the start of the treatment takes into account uterine involution (approximately 1 month) and the sexual dormancy caused by lactation (lactational anoestrus) which may superimpose itself on the seasonal anoestrus for winter-early spring lambing (3 months minimum from February to May).

Ewes were assigned to four experimental groups. *Group 1*, F_1 and F_2 cross Pleven Blackhead x Awassi. Adult lactating ewes were with body weight 45-50 kg. The animals were lambed during months of December, last year and January, present year. *Group 2*, F_1 and F_2 crosses Pleven Blackhead x Awassi. Adult lactating ewes were with body weight 45-55 kg. The animals were lambed during months of October - November, last year. *Group 3*, pure breed Awassi. Adult lactating ewes with body weight 42-48 kg. The animals were lambed during month of December, last year. *Group 4*, pure breed Ile de France. Adult non-lactating ewes with body weight 65-70 kg. The

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animals were lambed during month of November, last year.

Timing of melatonin treatment. Melovin® (CEVA Sante Animale, France) implants were inserted subcutaneously from 15th March to 5th of April. The treatment period was 35 days, before ram introduction. Melovine implanter was used to administer one implant subcutaneously near the base of the ear for ewes and 3 implants for every ram.

Breeding. Naturally "hand mating" with adult proven rams. Breeding period was 30 days from the beginning to the end. Data was collected during the whole experimental period and after the lambing.

Pregnancy diagnosis. Pregnancy diagnostics with ultrasound scanner CHIZON VET were performed in each group 30 days after the last mated ewes and finely after the lambing.

Statistical differences between fertility, lambing rate, and litter size were tested using Duncan's multiple range tests. Differences in occurrence of sex ratios among groups were analyzed by the Chi-square test. For all statistical analyses, the minimum significance level was $P < 0.01$.

Results and discussion

A significant effect of farm and time of melatonin treatment was recorded for fertility, litter size and fecundity in the three breeds under study (Table 1). Melatonin treatment increased the fertility rate significantly in the two spring treatment periods: March - ewes groups I and IV, and April – ewes group II in the Pleven Blackhead x Awassi and Ile de France flocks. Regarding litter size, the melatonin-treated ewes recorded a significantly higher number of lambs born after treatment in March in the meat breed. Lower result (115% fecundity) was obtained in experimental group III (Awassi). The melatonin implants improved the fertility in the ewes when inserted in spring (II - 94,5%; III – 93,26%; IV – 91,42%). The highest increment in the number of lambs born per ewes treated in these three breeds was obtained in April.

The most evident effect in the use of melatonin implants in the crosses F1 and F2 (Pleven Blackhead x Awassi) milking breed and Ile de France was the improvement of fecundity (133,3% instead 122,5%; 136,4%↔123%; 157%↔151%,- unpublished communications) in all treatment periods, except May. Although litter size was not affected by the use of the hormone, the results in these breeds showed a significant improvement in fecundity ($P<0.01$) after implantation in January and February according to data from previous investigations in Bulgaria (unpublished communications).

The improvement of litter size induced by melatonin after implantation in spring is mainly due to an increased ovulation rate (Table 2), as has been previously reported in both seasonal (Haresign, 1992) and Mediterranean ewes (Forcada et al., 1995; Zuniga et al., 2002). Some recent studies on synchronized ewes found melatonin treatment not to modify the timing of follicle emergence, but that it increases the number of ovulatory follicles by decreasing the atresia of medium and large follicles (Bister et al., 1999, Vazquez et al., 2009)

Considering the particular conditions on the farms where this trial was carried out implies that a farm effect should be considered. Melatonin was able to increase the overall number of lambs produced in the three breeds under study, although its efficacy seems to differ, considering the breed and time of treatment. Thus, it should be considered that the expected effects of melatonin can be achieved to a variable extent on commercial farms, provided that a realistic assessment of specific conditions on each farm is used to determine a treatment time. The most consistent results were

Table 1. Reproductive performance of Awassi, crosses Black Head x Awassi and Ile de France ewes treated with melatonin (Melovin) during the spring.

Groups	Month	Sheep treated (n)	Lambed	Fertility (%)	Lambs born	Treatment №
Group 1, Awassi crosses	March	100	74	74^{abc}	101	136.4^{d}
Group 2, Awassi crosses	April	200	189	94.5^{a}	252	133.3^{e}
Group 3, Awassi	March	104	97	93.26^{b}	112	115^{f}
Group 4, Ile de France	March	280	256	91.42^{c}	402	157^{def}

*****a, b, c, d, e, f – The same letters mark statistically significant differences between the groups (p<0, 001).

Table 2. Frequency of litter size in different sheep breeds after melatonin treatment during the spring.

Litter size*	Breeds			Total Frequency (%)
	Ile de France (%)	Awassi crosses (%)	Pure Awassi (%)	
0	8.57	5.5	6.7	5
1	40.35	67.5	78.8	57.964
2	50.0	25.5	14.5	36
3	1.08	1.5	0	1.036
				100

*Litter size: 0 – non lambed, 1 – lambed one, 2 – lambed two, 3 – lambed three obtained in the Ile de France breed, with good results both in winter and spring melatonin treatment. The best results in the F1 and F2 Awassi breed were obtained when melatonin implants were inserted in spring. It can be concluded that melatonin could be a useful tool to improve lamb production in the three breeds under study, although its efficacy within each breed seems to vary according to the farm and season.

The percentages of mated ewes which lambed were 74%, 94,5%, 93,26% and 91,42% in each group, respectively. The twinning rates of these groups (Table 2) were 50% for Ile de France, 25,5% for F1, F2 Awassi crosses and 14,5% for pure Awassi, respectively. Twinning rates were significantly higher in the first group ($P < 0.05$). In the group pure breed Awassi, to which melatonin was administered, a twinning rate lower than that of the Ile de France and Awassi crosses group was observed. Wellace et al., (1988) reported that melatonin increased the progesterone level by its luteotropic effect and this enhanced embryonic survival. Some other researchers have also pointed out that melatonin increased the twinning percentage by having a positive effect on pregnancy and embryo survival rates. However, since it is not possible to determine the ovulation rate, it is hard to determine whether this twinning effect is caused by increasing the ovulation rate or decreasing the early embryonic losses. Haresign et al., (1990) claim that melatonin increases the ovulation rate; in contrast, some other researchers state that the ovulation rate does not change but pregnancy and live fetus rates increase in the melatonin treated group. These researchers explain this by the effect of melatonin on corpus luteum and its increasing the progesterone level during the luteal phase and supporting embryo development.

Results in the present study show exogenous melatonin treatment to improve the production of lambs in the different Bulgarian breeds of sheep. However, results are different when either breed or dates of treatment are considered. In the present experiment, and in spite of the attempt to equalize farming conditions, farm and date effects were confounded, and the results need to be carefully analyzed and interpreted. Thus, in a trial designed to evaluate melatonin treatment on 21 commercial sheep farms in France, Chemineau et al. (1992) recorded significant farm and melatonin effects on the reproductive performance, but an absence of interaction between both parameters.

Table 3. Reproductive performance in ewes and sex ratio in new born lambs after treatment with Melovin.

Groups	Treated Lambed*	Lambs (n)	Male		Female		χ^2
			n	%	n	%	
Group 1, Awassi crosses	100 74*	101	56	55.40	45	44.60	1.16
Group 2, Awassi crosses	200 189*	252	123	48.80	129	51.20	0.45
Group 3, Awassi	104 97*	112	49	43.75	63	56.25	1.74
Group 4, Ile de France	280 256*	402	189	47.01	213	52.99	0.356

As initially determined by Short (1974), and later refined by Clarke et al. (1976), sexual differentiation in lambs occurs from approximately 30 to 100 days of the 145 day gestation. Therefore, our hypothesis herein was that a ewe's melatonin implantation would alter fetal organ development; specifically those organs associated with the GnRH axis, and would affect postnatal development.

There were no significant differences between male and female lambs after melatonin treatment, analyzing with χ^2 (Table 3). Physiologically, an altered sex ratio might be achieved by facilitating or inhibiting the transport of either X- or Y-bearing sperm through the reproductive tract or sex-specific death of embryos after fertilization (Hardy, 1997). William (1987) emphasized that in cases where artificial insemination is preceded by hormone treatment, the sex ratio (proportion of males) is lower and gonadotropin has a pre-conceptional sex-selective effect in humans. Overall result of this study has shown that sex ratio is not dependent on the melatonin treatment of ewes. Similarly, Haresign (1992) in an experiment involving 18 farms and two sheep breeds in the UK, found a considerable degree of variation between flocks in the number of extra lambs produced per ewe exposed to the ram. However, it was concluded that much of this could be accounted for by seasonal differences at the time of treatment. This could be the cause of the differences between farms observed in this experiment within breed, as ewes were treated from January (winter) to May (spring). In a third field trial carried out in Bulgaria, it was concluded that the potential of Bulgarian sheep to respond to melatonin treatment varies in relation to the breed, month and reproductive history of the farm (unpublished communication).

Melatonin treatment was effective in Awassi, Awassi crosses and Ile de France ewes after treatment in spring (February to April), on Farms 3 and 4, respectively, where 0.30 extra lambs per ewe treated were obtained. This improvement is very similar to that previously reported for the Spanish breed treated in March (spring), and is associated with a significant increase in both fertility and prolificacy (Forcada et al., 2002).

Conclusion

Reproductive technologies in animal husbandry significantly raise the level of production and genetic progress. The present studies have led to main conclusions regarding the sites of action of melatonin. Melatonin, given by the insertion of micro-implants, is able to stimulate LH secretion. These results show that Melovin micro-implants could stimulate LH secretion in the experimental animals when used during the month March and April. The response of ewes to melatonin treatment may vary according to breed, season, and the latitude where the study was conducted. Exogenous melatonin treatment could improve ovulation rate in ewes. The result in the present study showed that melatonin micro-implants treatment in pure Awassi breed did not affect litter size.

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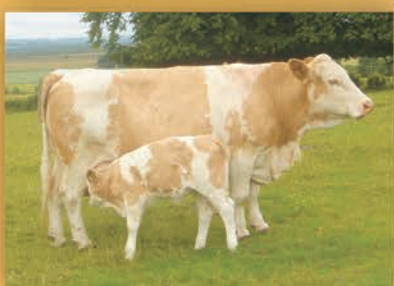

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